Available online on 15.09.2014 at http://jddtonline.info
Journal of Drug Delivery and Therapeutics
Open access to Pharmaceutical and Medical research

RESEARCH ARTICLE

EVALUATION OF PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF SEEDS USED IN TRADITIONAL MEDICINE

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ABSTRACT

Phytochemicals are secondary metabolites of plants which are non-nutritive chemicals that have protective or disease preventive properties. It is well known that plants produce these metabolites to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. The seeds of Cucumis sativus, Citrullus lanatus and Cucurbita pepo are used in herbal medicine as such or in the form of seed oil. They have various medicinal properties. Qualitative and quantitative analysis of phytochemicals and their antioxidative property is evaluated. Antioxidants are beneficial in oxidative stress related diseases. Acetone extracts of all the three seeds showed the presence of phenols, flavonoids and carbohydrates. Saponins, alkaloids, steroids, tannins and anthocyanins were absent in all the three extracts. Citrullus lanatus seeds with 7.5mg and 92.6mg GA equivalent/g of extract powder showed maximum amount of Phenolics and flavonoids. The total sugars and proanthocyanidins was maximum in Cucurbita pepo seeds with 3.6 µg glucose equivalent/g of extract powder and 171mg GA equivalent/g of extract powder. Citrullus lanatus seed extract showed the highest reducing power activity, Free radical scavenging potential and inhibition of lipid peroxidation.

Key Words: Phytochemicals, Antioxidant activity, Lipid peroxidation, Reducing power activity, Cucumis sativus, Citrullus lanatus and Cucurbita pepo

INTRODUCTION

The major phytochemicals from medicinal plants and their seeds, fruits and vegetables which are in extensive study for pharmacological actions are polyphenols derived from carbohydrates, terpenes from a group of lipids and alkaloids from amino acids. Several antioxidants from the above phytochemicals are known to act as anti-inflammatory compounds [1, 2]. Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Oxidative stress is an imbalanced state where excessive quantities of reactive oxygen and/or nitrogen species (ROS/RNS, e.g., superoxide anion, hydrogen peroxide, hydroxyl radical, and peroxynitrite) overcome endogenous antioxidant capacity, leading to oxidation of varieties of biomacromolecules, such as enzymes, proteins, DNA and lipids. Oxidative stress causes the development of chronic degenerative diseases including coronary heart disease, cancer and ageing. Recently, phenolics have been considered powerful antioxidants *in vitro* and proved to be more potent antioxidants than Vitamin C and E and carotenoids. It has been proposed that the antioxidant properties of phenolic compounds can be mediated by the following mechanisms: (1) scavenging radical species such as reactive oxygen species/ reactive nitrogen species ROS/RNS, [2] Suppressing ROS/RNS formation by inhibiting some enzymes or chelating trace metals involved in free radical production,[3] upregulating or protecting antioxidant defense. Due to some common side effects of synthetic drugs (NSAIDs and steroidal) such as gastric irritation, ulceration, bleeding, renal failure, interstitial nephritis, hepatic failure, headache, thrombocytopenia, hemolytic anaemia, asthma exacerbation, skin rashes, and angioedema, Its use is considered to be unsafe. Hence, treatment of inflammatory diseases by herbal drugs has keen interest to the researchers. From the study made globally, it has been known that the market for use of herbal drugs in the treatment of inflammatory diseases constitutes 83% worldwide and is expected to reach a value of around more than 95% in the forthcoming years due to increased acceptability of these preparations. In India, the use of different parts of several medicinal plants to cure specific ailments has been in vague from ancient times. Therefore, now there is a need to look back towards the traditional medicine which can serve as novel therapeutic agent. Many oxidative stress related diseases such as diabetes, aging, and other degenerative diseases in humans are as a result of accumulation of free radicals in the body [3]. A lot of researches are going on worldwide directed towards finding natural antioxidants of plants origins.

The aim of this study was to screen Cucumis sativus, Citrullus lanatus and Cucurbita pepo seed acetone extract for their phytochemical constituents, quantification of

those phytochemicals and their antioxidant activity. As a result various new antioxidants identified from seeds are screened for antioxidant activity which has potential therapeutic application.

Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities [4]. The antioxidant activity is mainly due to the presence of phytochemicals such as phenolics (gallic acid, ellagic acid, ferulic acid, tannic acid, etc), flavonoids (flavonols, flavones, flavanones, flavanonol, flavan-3-ols, biflavonoids, prenylated flavonol and synthetic flavone) and anthocyanins (delphinidin, cyanidin, pelargonidin, peonidin, petunidin, malvidin, etc). Active research has been driven in recent years on plant components due to their biologically beneficial effects emanating from antioxidant activities of phenolic phytochemicals.

MATERIALS AND METHODS

Collection of plant material:

The dried seeds of *Cucumis sativus*, *Citrullus lanatus* and *Cucurbita pepo* were purchased from the local market. The seeds were cleaned, powdered and stored in airtight containers until further studies.

Preparation of Extract:

10 g of each powder was extracted in 20ml of acetone by stirring using a magnetic stirrer at cold condition for 4 hours. The extracts were centrifuged at 10,000rpm for 10 minutes and then filtered through Whatman no.1 filter paper for removal of particulates. The residues obtained were re extracted with another 20ml of acetone and again the process was repeated. The acetone extracts of different parts were pooled separately and concentrated under vaccum at 40°C. The yield was calculated and expressed as percentage of w/w.

Table 1: Yield of *Cucumis sativus*, *Citrullus lanatus* and *Cucurbita pepo* seed extracts.

S.No	Extracts	Yield (%W/W)
1	*Cucumis sativus*	**33.7**
2	*Citrullus lanatus*	**34.6**
3	*Cucurbita pepo*	**38.5**

Preliminary phytochemical screening:

A small portion of the dry extract was used for the phytochemical tests for compounds which include alkaloids, carbohydrates, flavonoids, tannins, anthocyanins, steroids, phenols and saponins in accordance with the methods of with little modifications [5, 6]. Exactly 1.0 g of plant extract was dissolved in10 ml of distilled water and filtered (using Whatman No 1 filter paper) A blue colouration resulting from the addition of ferric chloride reagent to the filtrate indicated the presence of tannins in the extract. Exactly 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl on steam bath. A milliliter of the filtrate was treated with few drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloid. About 0.2 g of the extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange colouration was indicative of the flavonoids.

TABLE 2: THE PHYTOCHEMICAL ANALYSIS OF ACETONE EXTRACTS OF SEEDS

Sl No	Tests	*Cucumis sativus*	*Citrullus lanatus*	*Cucurbita pepo*
1	**Alkaloids**			
	Mayers test	-	-	-
	Wagners test	-	-	-
	Dragendroffs test	-	-	-
2.	**Carbohydrates**			
	Molisch tests	+	+	+
	Fehling test	+	+	+
	Benedict test	+	+	+
3.	**Flavonoids**			
	Shinoda test	+	+	+
	Lead acetate test	+	+	+
	Alkaline reagent test	+	+	+
4.	**Saponins**			
	Foam test	-	-	-
	Froath test	-	-	-
5.	**Steriods**			
	Salkowski's test	-	-	-
6.	**Tannins**			
	Gelatin test	-	-	-
7.	**Anthocyanins**	-	-	-
8.	**Phenols**			
	Ferric chloride test	+	+	+

 ISSN: 2250-1177 CODEN (USA): JDDTAO

Freshly prepared 7% blood agar plate was used and wells were made in it. The crude extract dissolved in 10% methanol was used to fill the wells bored in the blood agar plates. Ten percent methanol was used as a negative control while commercial saponins solution was used as a positive control. The plates were incubated at 35°C for 6 h. complete hemolysis of the blood around the extract was indicative of saponins. About 0.5 g of the extract was dissolved in 3 ml of chloroform and filtered. Concentrated H_2SO_4 was carefully added to the filtrate to form lower layer. A reddish brown colour at the interface was taken as positive for steroid ring.

DETERMINATION OF TOTAL PHENOLICS BY FOLIN_CIOCALTEAU ASSAY:

The concentration of total Phenolics in the acetone extracts of *Cucumis sativus, Citrullus lanatus and Cucurbita pepo* seeds was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium[7]. The phenolic contents of the extracts were determined from calibration curve and were expressed in mg gallic acid equivalent/g of extract powder.

DETERMINATION OF TOTAL SUGARS BY PHENOL-SULPHURIC ACID METHOD:

Carbohydrate content of all the extracts at 100µg concentration was determined by the phenol-sulphuric acid method.

ESTIMATION OF TOTAL FLAVONOIDS

Aluminum chloride colorimetric method was used for flavonoids determination [8]. The content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg gallic acid equivalent/g of extract powder

DETERMINATION OF TOTAL PROANTHOCYANIDINS:

Total proanthocyanidin was determined for all the three seed acetone extracts based on the procedure of Sun et al [9]. Total proanthocyanidin content was expressed as gallic acid equivalent (mg/g) from the standard curve.

DETERMINATION OF REDUCING POWER:

The reducing power of all the three acetone seed extracts was evaluated according to the method of Oyaizu [10].

ANTIOXIDANT ACTIVITY BY DPPH METHOD:

2, 2-Diphenyl -1- picrylhydrazyl radical ($DPPH^{\cdot}$) was used as a stable radical for assessing antioxidant activity as described by Blios[11]. Reduction of $DPPH^{\cdot}$ by an antioxidant or by a radical species results in a loss of absorption at 517nm. Thus the degree of discoloration of the solution indicates the scavenging efficiency of the added substances. Determination of antioxidant activity by the DPPH method was done for all the three acetone seed extracts at 100µg concentration. Changes in the absorbance of the samples were measured at 517 nm.Radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula:

Percentage of radical scavenging activity = Control OD-sample OD/Control OD

ANTIOXIDANT ACTIVITY BY TBA METHOD:

Antioxidant activity of all three acetone seed extracts was performed using thio barbituric acid (TBA) according to the protocol of Halliwell and Gutteridge[12]. Lipid peroxidation induced by ferric chloride resulted in the production of malondialdehyde (MDA), lipid peroxide. Thio-barbituric acid (TBA) reacts with malondialdehyde (MDA) to form a di-adduct, a pink chromogen, which can be detected spectrophotometrically at 532nm.

Table 3: Total phenolic, sugar, flavonoids and proanthocyanidins of *Cucumis sativus, Citrullus lanatus* and *Cucurbita pepo* seeds extracts.

Extracts	phenolic	sugar	flavonoids	proanthocyanidins
Cucumis sativus	3.8±0.2	3.0±0.2	75.4±0.6	166±0.9
Citrullus lanatus	7.5±0.3	2.8±0.4	92.6±0.6	86±0.8
Cucurbita pepo	0.962±0.1	3.6±0.0.5	11.34±0.4	171±0.5

Data represents mean ±SD (n=3).

 ISSN: 2250-1177 CODEN (USA): JDDTAO

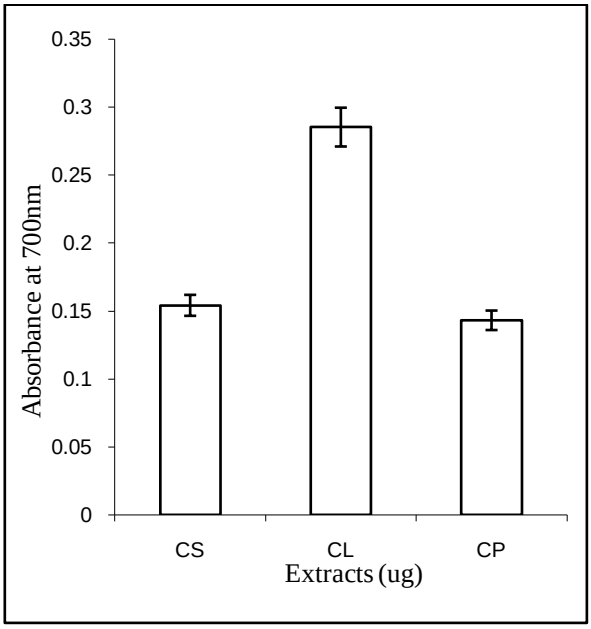

Figure 1: Reducing power activity in acetone extracts of *Cucumis sativus* (CS), *Citrullus lanatus* (CL) and *Cucurbita pepo* (Cp) seeds. Data represents mean ±SD (n=3).

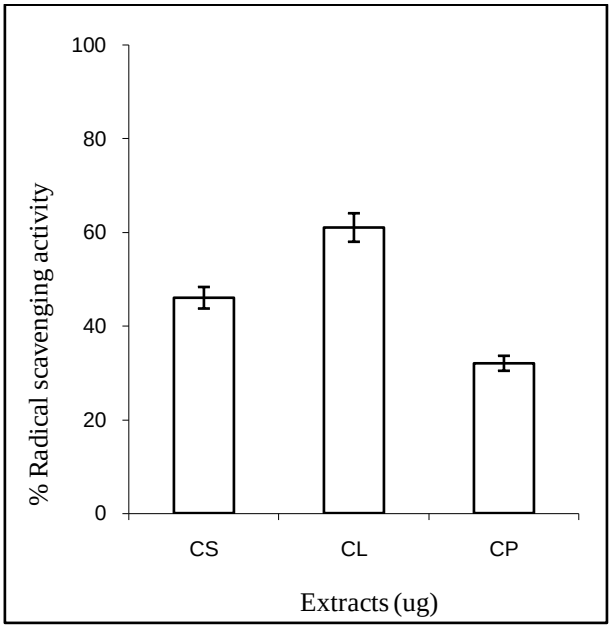

Figure 2: Antioxidant activity by DPPH method in acetone extracts of *Cucumis sativus* (CS) , *Citrullus lanatus* (CL) and *Cucurbita pepo* (Cp) seeds Data represents mean ±SD (n=3).

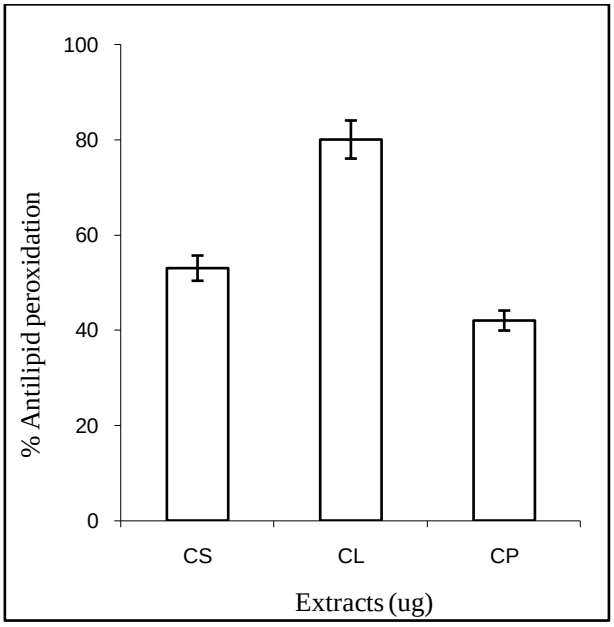

Figure 3: Antioxidant activity by TBA method in acetone extracts of *Cucumis sativus* (CS), *Citrullus lanatus* (CL) and *Cucurbita pepo* (Cp) seeds. Data represents mean ±SD (n=3).

RESULTS AND DISCUSSION:

The plant products over synthetic compound in the treatment of diseases are needed because of no deleterious effects on man. India is a home to a variety of traditional medicine system that relay to a very large extent on native plant species for their raw drug materials. Therefore there is a need to look backwards towards folk medicines which can serve as novel therapeutic agent. Many secondary metabolites grouped under polyphenols, terpenes and alkaloids exhibit antioxidant activity. The free radical intermediates and ROS escape from the site of reaction and act on various biological molecules such as lipids, nucleic acids, proteins and carbohydrates, thus causing deleterious changes in their structure and function and finally leading to cell death[13].

Cucumis sativus, Citrullus lanatus and *Cucurbita pepo* seeds were purchased from market. The dried seeds were cleaned, and powdered. The powders in the range of 10 g was used for extraction using acetone. They were

extracted on a magnetic stirrer for fixed amount of time. After extraction the extracts were centrifuged at 10,000 rpm for 10 minutes and the supernatant obtained was filtered through whatman No.1 filter paper. The residue left behind was re-extracted with another 20 ml of solvents and filtered. The filtrates obtained from all extracts were pooled and concentrated under vacuum at 40 ^{o} C The percentage of yield (w/w) was calculated for all acetone extracts as depicted in Table 1. The yield obtained from these extracts ranged from 33.7 to 38.5% The yield was found to be greater in *Cucurbita pepo* seeds followed by *Cucumis sativus* and *Citrullus lanatus* seeds respectively.

Different phytochemicals have various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well being. The qualitative analysis of the acetone seed extracts of *Cucumis sativus, Citrullus lanatus* and *Cucurbita pepo* showed the presence of phytochemical constituents such as carbohydrates, flavonoids, and phenolics. At the same time, the phytochemical constituents such as saponins, steroids, anthocyanins and tannins were found to be absent as tabulated in Table 2.

Phenolic compounds are naturally occurring secondary metabolites that are of great pharmacological interest[14].Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Total phenolics was estimated for all the three acetone seed extracts of *Cucumis sativus, Citrullus lanatus* and *Cucurbita pepo* fruits at 100 µg concentration as shown in Table 3. Out of the 3 acetone seed extracts, acetone extract of water melon with 7.5 mg gallic acid equivalent/g of extract powder showed maximum amount of phenolics followed by the *Cucumis sativus* and *Cucurbita pepo* seeds with 3.8 and 0.962 mg gallic acid equivalent/g of extract powder. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties. They are considered as major determinant of the antioxidant activity of nuts and plants.

The total carbohydrate contents of all the three seed extracts are presented in Table 3. The acetone seed extracts of *Cucumis sativus, Citrullus lanatus* and *Cucurbita pepo* was used for total sugars estimation at 100 µg concentration The acetone extract of *Cucurbita pepo* seed with 3.6 ug glucose equivalent/g of extract powder showed maximum amount of sugars followed by the and *Cucumis sativus* and *Citrullus lanatus* seeds with 3.0 and 2.8 ug glucose equivalent/g of extract powder..

Flavonoids are the most abundant polyphenols in our diets. Flavonoids a group of polyphenolic compounds with known properties such as free radical scavenging activities, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory activities have been isolated from plants. Therefore the total flavonoid content was estimated in all the three seed extracts. The total flavonoid content of *Cucumis sativus, Citrullus lanatus* and *Cucurbita pepo* seed extracts was 75.4, 92.6 and 11.34 mg gallic acid equivalent/g of extract powder as shown in Table 3 with *Citrullus lanatus* acetone seed extract maximum followed by *Cucumis sativus* and *Cucurbita pepo* seed extracts. These results gives a reason for the activity of these plants as antioxidant and how these plants extracts enable to scavenge the free radicals

proanthocyanidins serve among other chemical and induce defense mechanisms against plant pathogens and predators, such as in strawberries[15]. The proanthocyanidin content was determined for all the three acetone seed extracts and the results were as shown in Table 3.Pumpkin seed with 171 mg gallic acid equivalent/g of extract powder was found to be high in proanthocyanidins followed by *Cucumis sativus* and *Citrullus lanatus* seeds with 166 and 86 mg gallic acid equivalent/g of extract powder

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Figure 1 shows the reducing power activity of *Cucumis sativus, Citrullus lanatus* and *Cucurbita pepo* seed extracts at 100 µg concentrations using the potassium ferricyanide reduction method. At 100 µg concentration, the extracts showed absorbances of 0.154, 0.285 and 0.143 respectively at 700 nm. Thus, the highest reducing activity was observed in *Citrullus lanatus* acetone seed extract. The reducing power activity is due to the presence of reductones (phenolics).

Free radical scavenging potentials of all the three acetone seed extracts at 100 µg concentration were tested by the DPPH method and the results are shown in Figure2 *Cucumis sativus, Citrullus lanatus* and *Cucurbita pepo* seed extracts exhibited 46.0%, 61.0% and 32.0 % free radical scavenging activity respectively according to the DPPH method. . DPPH is a stable radical that has been used to evaluate the antioxidant activity of rhizome extract.. Antioxidant reacts with DPPH, which is a stable free radical, and convert it to α, α-diphenyl-β-picryl hydrazine. The degree of discolouration indicates the scavenging potentials of the antioxidant extract. The activity of extracts is attributed to their hydrogen donating ability. Increasing the number of hydroxyl or catechol groups increases radical scavenging activity. In presence of other H-donating groups (sulfhydryl, amide) in molecule also accelerates this activity.

The results of the effect of various seed extracts at 100 µg concentration to prevent lipid peroxidation are shown in Figure.3. At 100 µg concentration, the *Cucumis sativus, Citrullus lanatus* and *Cucurbita pepo* seed extracts showed 53%, 80 % and 42 % of inhibition of lipid peroxide generation by this method. Determination of the lipid peroxide content was carried out indirectly by means of derivatizing MDA with TBA at high temperature and acidic conditions. . In biological systems, MDA is a very reactive species and takes part in cross-linking of DNA with proteins and also damages the liver cells Therefore, inhibition of lipid peroxidation is of great importance in the PLA_2 mediated inflammatory disease processes involving free radicals. The production of lipid peroxides by ferrous/ascorbate systems in liver

homogenates were inhibited greatly by the acetone extract of *Cucumis sativus, Citrullus lanatus* and *Cucurbita pepo* seeds Increased PLA_2 activity and arachidonic acid mobilization has been reported under conditions promoting increased cell or tissue lipid peroxidation. If radicals initiate lipid peroxidation and PLA_2 activation, then the ability of antioxidants to scavenge radicals within the hydrophobic core of the membrane may explain their desirable therapeutic effect.

CONCLUSION:

Thus acetone seed extract of *Citrullus lanatus* had maximum amount of polyphenolics and flavonoids which is directly related to their greater antioxidant activity. Further isolation of molecules from the extract can be used to inhibit enzymes responsible for free radical generation. It may be useful in targeting various metabolic pathways necessary for disease prevention.

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 ISSN: 2250-1177 CODEN (USA): JDDTAO